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(21) International Application Number: PCT/EP96/02718 (22) International Filing Date: 21 June 1996 (21.06.96) (30) Priority Data: MI95A001367 27 June 1995 (27.06.95) IT (71) Applicant (for all designated States except US): INDENA S.P.A. [IT/IT]; Viale Ortles, 12, I-20139 Milano (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): BOMBARDELLI, Ezio [IT/IT]; Via Ortles, 12, I-20139 Milano (IT). GABETTA, Bruno [IT/IT]; Viale Ortles, 12, I-20139 Milano (IT). (74) Agent: MINOJA, Fabrizio; Studio Consulenza Brevettuale, Via Rossini, 8, I-20122 Milano (IT).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: COLCHICINE DERIVATIVES, THE USE THEREOF AND FORMULATIONS CONTAINING THEM (57) Abstract <p>The present invention relates to novel colchicine derivatives having antiproliferative, antineoplastic, anti-inflammatory and muscle relaxant activities; said derivatives include novel colchine nitrogen amides for use either as such or after derivatization of the hydroxyl at C₃ of the aromatic ring and at C₁₀ of the tropolone ring. These novel compounds have a cytotoxicity on human tumoral cell lines comparable with colchicine but, in comparison with the latter, they are much more active on cells resistant to the usual antineoplastics. The compounds can be included in pharmaceutical formulations useful for the intravenous, oral and topical administrations.</p>		

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COLCHICINE DERIVATIVES, THE USE THEREOF AND FORMULATIONS
CONTAINING THEM

The present invention relates to novel colchicine derivatives having antiproliferative, antineoplastic, antiinflammatory and muscle relaxant activities, the methods for the preparation thereof and the pharmaceutical formulations containing them.

Colchicine is a known pseudo-alkaloid widely used for a very long time in therapy for the treatment of gout, a pathology on which it acts very quickly and specifically, even though it should be used for short times due to its toxicity. A colchicine derivative, namely thiocolchicoside, is widely used to treat contractures and in inflammatory conditions on skeletal muscles. In addition, colchicine is a very potent antiproliferative agent, which acts blocking the formation of the mitotic spindle during cell division; this latter aspect has been investigated thoroughly for any antineoplastic activity and a great deal of colchicine derivatives have been prepared to this purpose. Colchicine as such and a number of its derivatives could not be used clinically due to their high toxicity, and therefore their unacceptable risk/benefit ratio. Only one colchicine derivative, demecolcine, is used in some degree in oncology for the treatment of some leukemia forms. The products of the invention differ from those of the prior art in their high activity, lower toxicity and higher therapeutical index. More specifically, in the antineoplastic field, researches have been focused on the search for products having, besides a normal

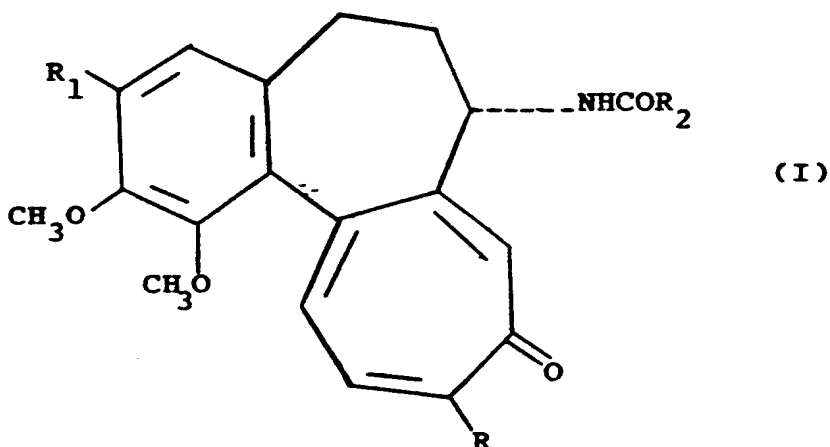
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cytotoxicity, a cytotoxicity aimed at cell lines resistant to the known, usual antitubercular medicaments.

The derivatives of the present invention have the formula I:

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wherein R is a methoxyl or thiomethyl group;

15 R_1 is hydroxy; a B-D-glucopyranosyloxy residue; a B-D-glucopyranosyloxy residue ketalized at the hydroxyls 4' and 6' with aliphatic or aromatic aldehydes; a 6-deoxy-galactopyranosyloxy residue; an acyloxy group of C_{16} to C_{22} polyunsaturated fatty acids; straight, branched or
20 cyclic O-alkyl C_1 - C_6 , saturated or unsaturated; and R_2 is a C_1 - C_6 haloalkyl group.

Particularly preferred compounds of formula I are those in which R_1 is a methoxy group, a B-D-glucopyranosyloxy residue optionally ketalized at the 4' and 6' hydroxyls with aromatic or aliphatic aldehydes,
25 for example, 2- or 3-thienal or a ximenoyloxy group.

R_2 is preferably trifluoromethyl, pentafluoroethyl or heptafluoropropyl.

Compounds I are prepared starting from the natural
30 compounds colchicine or thiocolchicine (Formula I, R_1 = $-OCH_3$, R_2 = CH_3 , R = $-OCH_3$ or $-SCH_3$, respectively) or

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from the corresponding derivatives thereof glucosylated at the hydroxyl at the 3- position or also from the N-formyl-N-deacetyl-derivatives thereof.

The hydrolysis of said natural compounds with aqueous solutions of strong mineral acids makes it possible to obtain selectively, changing the temperature and the reaction time, the corresponding N-deacetyl and 3-demethyl-N-deacetyl derivatives which can then be subjected to conventional reactions of N-acylation and alkylation or acylation at the hydroxyl at the 3-position.

In the case of thiocolchicine, the hydrolysis with hydrohalogen acids or, more preferably, with sulfuric acid (20% H_2SO_4 - 120 h) allows to obtain N-deacetylthiocolchicine and 3-demethyl-N-deacetylthiocolchicine in nearly quantitative yields.

The compounds of the invention have a remarkable antitumour activity.

The table shows the antimitotic activity of the compounds of the invention on a cultured breast tumour explant, compared with colchicine and Taxol.

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Table

In vitro cytotoxic activity of some thiocolchicine derivatives.

5	Compounds	IC ₅₀ (nM)		
		MCF7-ADR (resistant)	MCF-7 (human breast)	MCF7-ADR/MCF7
	Colchicine	112 ± 4.2	4.4 ± 0.3	25.45
10	Compound I	26 ± 2.3	6.2 ± 0.4	4.2
	Compound I	11 ± 1.9	5.0 ± 0.2	2.2
	Compound III	7 ± 0.4	4.1 ± 0.3	1.7
	Compound IV	31 ± 1.9	3.2 ± 0.2	9.7
	Taxol	360 ± 7.8	6.1 ± 0.3	59.01

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This table evidences that the compound of the invention have significant advantages on the resistant cell lines, which are nowadays considered the main target for cytotoxic medicaments.

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Moreover, the products according to the present invention have antiinflammatory and muscle relaxant activities and they can be incorporated in pharmaceutical formulations useful for the administration of the medicament for the indicated

25 pathology. Formulations for the intravenous, oral, transdermal, epicutaneous administrations can conveniently be prepared.

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Among the excipients useful to prepare said formulations, natural and synthetic phospholipids proved to be particularly useful for preparing liposomal forms for the parenteral, intravenous and/or topical routes.

The same formulations proved to be useful in the topical treatment of cutaneous epitheliomas and in cutaneous hyperproliferative conditions, such as psoriasis. In the specific antineoplastic field, besides the phospholipids which allow the administration of the medicament in the liposomal form, some surfactants such as polyethoxylated castor oils, or polysorbates acting synergistically with the active ingredient, turned out to be particularly useful. Preferably the active principle is micronized until the compound is dissolved in water. In oncology, the products are used at dosages from 1 to 100 mg/m².

The following examples further illustrate the invention.

Example I - Preparation of N-deacetyl-N-pentafluoro-propionyl-thiocolchicine. (Compound I; R = -SCH₃ R₁ = -OCH₃ R₂ = -CF₂-CF₃)

20 g of thiocolchicine are dissolved in 300 ml of 20% sulfuric acid and heated under nitrogen atmosphere for 36h at 100°C; the reaction mixture is alkalized to pH 8 to separate 15 g of N-deacetyl-thiocolchicine.

This product is dissolved in acetone and, in the presence of anhydrous Na₂CO₃, it is reacted with 1.5 equivalents of perfluoropropionic anhydride under strong stirring; after 2h the reaction mixture is filtered and the solvent is evaporated off. The oily residue is taken up with methanol, from which N-deacetyl-N-pentafluoro-propionylthiocolchicine is separated by crystallization.

Example II - Preparation of N-deacetyl-N-pentafluoro-propionyl-3-O-ximenoyl-thiocolchicine. (Compound II; R = -SCH₃ R₁ = -O-Ximenoyl R₂ = -CF₂-CF₃)

20 g of thiocolchicoside are dissolved in 300 ml of 20% sulfuric acid and the whole is heated under nitrogen atmosphere 36h at 100°C; from the reaction mixture 12 g of N-deacetyl-3-O-demethylthiocolchicine separate.

5 This product is dissolved in acetone and, in the presence of anhydrous Na_2CO_3 , is reacted with 3 equivalents of perfluoropropionic anhydride under strong stirring; after 2h the reaction mixture is filtered and the solvent and the reactive excess are removed under vacuum. The residue consisting of N-deacetyl-N-pentafluoropropionyl-3-O-demethyl-3-O-pentafluoropropionate is taken up with methanol containing NH_4Cl , checking the hydrolysis of the phenol ester by thin layer chromatography (toluene/ethyl acetate 1:1); the solvent is evaporated to dryness under vacuum and the residue is dissolved in acetone, filtering off the insolubles. The acetone solution is concentrated to dryness and the residue is taken up with 100 ml of pyridine; this solution is cooled at 0°C and added with 2 eq. of ximeninic acid chloride under strong stirring. The reaction mixture is left to stand overnight and then poured onto 500 g of ice. The formed aqueous suspension is extracted for three times with 500 ml of methylene chloride. The organic phase is washed with water, then with a hydrochloric acid diluted solution and again with water. The phase organic is dried over Na_2SO_4 and concentrated to dryness. The residue is crystallized from an ethyl acetate/ isopropyl ether mixture, to obtain 27 g of N-deacetyl-N-pentafluoropropionyl-3-O-ximenoyl-thiocolchicine.

Example III - Preparation of N-deacetyl-N-pentafluoro-

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propionyl-3-O-demethyl-3-O-cyclopentenyl-thiocolchicine.
(Compound III; $R = -SCH_3$ $R_1 = -O-cyclopentenyl$
 $R_2 = -CF_2-CF_3$)

20 g of thiocolchicoside are dissolved in 300 ml of
5 20% sulfuric acid and the mixture is heated under
nitrogen atmosphere for 36h at 100°C; 12 g of N-
deacetyl-3-O-demethylthiocolchicine separate from the
reaction mixture.

This product is dissolved in acetone in the
10 presence of anhydrous Na_2CO_3 and reacted with 3
equivalents of pentafluoropropionic anhydride under
strong stirring; after 2h the reaction mixture is
filtered and the solvent and reactive excess are removed
under vacuum. The residue consisting of N-deacetyl-N-
15 pentafluoro-propionyl-3-O-demethyl-3-O-pentafluoropro-
pionate is taken up with methanol containing NH_4Cl ,
checking the hydrolysis of the phenol ester by thin
layer chromatography (toluene/ethyl acetate 1:1); the
solvent is evaporated to dryness under vacuum and the
20 residue is dissolved in acetone filtering off the
insolubles. The acetone solution is added with Na_2CO_3
and 5 equivalents of cyclopentenyl bromide with respect
to starting product. The reaction is stirred for 6h
checking the alkylation by thin layer chromatography.
25 When the reaction is complete, salts are filtered off
and the solvent is distilled under vacuum. The residue
is chromatographed on a silica gel column using ethyl
acetate as eluent. The fractions containing the desired
product are collected, solvent is removed and the
30 product is crystallized from acetone/hexane. 9.2 g of N-
deacetyl-N-pentafluoropropionyl-3-O-cyclopentenyl-thio-

colchicine are obtained.

Example IV - Preparation of N-deacetyl-N-heptafluorobutyroyl-thiocolchicine. (Compound IV; $R = -SCH_3$
 $R_1 = -OCH_3$ $R_2 = -CF_2-CF_2-CF_3$)

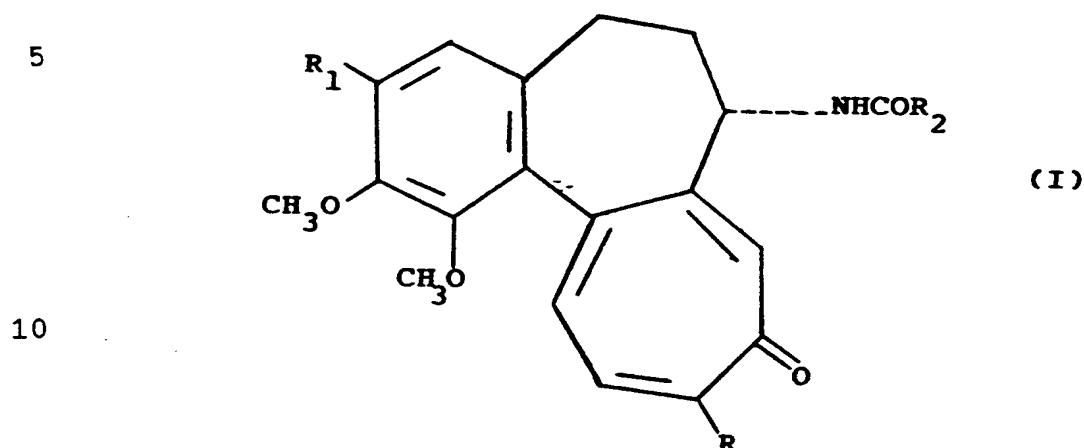
5 10 g of N-deacetylthiocolchicine are dissolved in
150 ml of anhydrous acetone in the presence of Na_2CO_3
and treated at room temperature with 1.5 eq. of
heptafluorobutyroyl anhydride. Na_2CO_3 and the solvent
are removed and the residue is purified with isopropyl
10 ether to give 12.5 g of N-deacetyl-heptafluoro-butyroyl-
thiocolchicine.

Example V - Preparation of N-deacetyl-N-pentafluoropropionyl-3-O-isopropyl-thiocolchicine. ($R = -SCH_3$ $R_1 = -O$ -
isopropyl $R_2 = -CF_2-CF_3$)

15 For the preparation of this derivative, the
procedures of example III are repeated, using isopropyl
bromide as the reagent. After purification of the crude
reaction product on silica gel and crystallization, 7.6
g of N-deacetyl-N-pentafluoropropionyl-3-O-isopropyl-
20 thiocolchicine are obtained, having spectroscopic
characteristics in agreement with the desired molecule.

CLAIMS

1. Compounds of formula I



wherein R is a methoxyl or thiomethyl group;

R_1 is hydroxy; a B-D-glucopyranosyloxy residue; a B-D-glucopyranosyloxy residue ketalized at the 4' and 6' hydroxyls with aliphatic or aromatic aldehydes; a 6-deoxy-galactopyranosyloxy residue; an acyloxy group of C_{16} to C_{22} polyunsaturated fatty acids; straight, branched or cyclic O-alkyl C_1 - C_6 , saturated or unsaturated; and R_2 is a C_1 - C_6 haloalkyl group.

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2. Compounds according to claim 1, wherein R_1 is a methoxy group, a B-D-glucopyranosyloxy residue optionally ketalized at the 4' and 6' hydroxyls with aliphatic or aromatic aldehydes selected from 2- or 3-thienal or a ximenoyloxy group.

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3. As compounds according to claim 1:

N-deacetyl-N-pentafluoro-propionyl-thiocolchicine;

N-deacetyl-N-pentafluoro-propionyl-3-O-ximenoyl-thiocolchicine;

30 N-deacetyl-N-pentafluoropropionyl-3-O-demethyl-3-O-cyclopentenyl-thiocolchicine;

N-deacetyl-N-heptafluoro-butyroyl-thiocolchicine;
N-deacetyl-N-pentafluoropropionyl-3-O-isopropyl-thiocolchicine.

4. Pharmaceutical compositions containing as the
5 active ingredient a compound of claim 1 in admixture
with suitable carriers or excipients.
5. Compositions according to claim 4, wherein the
active ingredient is formulated in liposomes.
6. Compositions according to claim 4, wherein
10 surfactants selected from polyethoxylated castor oils
and polysorbates are used.
7. The use of the compounds of claim 1 for the
preparation of antineoplastic, antiproliferative, anti-
inflammatory and muscle relaxant medicaments.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/02718

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07H15/248 C07C233/32 C07C323/41 A61K31/70 A61K31/645

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07C A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 34, 1991, WASHINGTON US, pages 3334-3338, XP002020104 RINGEL I. ET AL: "Fluorinated Colchinoids: Antitubulin and Cytotoxic Properties" see page 3334 - page 3335 ---	1,2,7
X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 33, no. 2, 1990, WASHINGTON US, pages 567-571, XP002020105 MUZAFFAR A. ET AL: "Antitubulin Effect of Derivatives of 3-Demethylthiocolchicin, Methlythio Ethers of Natural Colchinoids and Thioketones Derived from Thiocolchicine. Comparison with Colchinoids" see page 568 --- -/-	1,2,7

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIEBIGS ANNALEN DER CHEMIE, vol. 758, 1972, WEINHEIM, DE, pages 185-189, XP002020106 LETTRE H ET AL: "Synthese neuer Colchicin-Derivate mit hoher antimitotischer Wirksamkeit" see page 186 -----	1,2,7